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REVERSE-MUTATION AND ADAPTATION IN LEUCINELESS NEUROSPORA

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Mutations affecting specific steps in biochemical syntheses have been produced in *Neurospora crassa* by x-rays and ultra-violet radiation.[1] In crosses with wild-type molds, these mutations segregate in a Mendelian fashion and hence involve the alteration of single determinant factors. Since many of the mutants seem to completely lack a specific synthetic ability it was not certain whether they involved small chromosomal aberrations such as deficiencies, or the inactivation of genes which still self-duplicate. Up to the present time no demonstration of back-mutation, which would afford indirect evidence on this question, has been made for any of the biochemical mutants of *Neurospora*. On the basis of the evidence presented below we conclude that reverse-mutation to the wild-type allele does occur.

Strain 33757, which originated from a culture treated with ultra-violet light, is unable to synthesize the amino acid, leucine, an inability which has been shown by Regnery[2] to be caused by a difference from wild type in a single factor. In the course of the development of an assay method for leucine by the use of strain 33757 it was noted that the weights of some cultures were unusually high and did not bear the typical relationship to leucine in the medium which characterizes the growth of this mutant strain. The phenomenon was called adaptation.[3] Genetic and physiological studies have been made on three independently adapted strains of 33757.[4] Adapted strains α and β were derived as follows from a leucineless albino-marked stock of mating type *A* (33757-4637-*A*). Conidia were inoculated into flasks containing 0.25 mg. *l* (+) leucine per 50-ml. medium and were incubated at 30°C. for $8^1/_2$ days. Samples were taken from two cultures which subsequently proved to have unusually high weights, and inoculated into minimal medium. From this time, adapted cultures α and β were

subcultured on minimal medium where they grew like wild type. The third adapted strain, γ, was obtained from a salmon-colored leucineless stock of mating type a (33757-a) which was heavily inoculated into minimal medium. In one instance growth was observed and, upon subculturing, this adapted strain was prototrophic[b] and grew like wild type.

It should be emphasized that marker genes, for color and for mating type, were not modified in the course of adaptation. Furthermore, among more than 150 adaptations of the leucineless albino stock which we have observed in our studies, none have shown pigmentation. Consequently it is concluded that the adapted strains did not arise through contamination.

Genetic Studies.—In order to test the presumption that adaptation involves a genic mutation, the adapted cultures were first crossed with a leucineless strain of the opposite mating type. Asci were dissected and the spores allowed to germinate on medium containing leucine. The resulting strains were then tested for their ability to grow on minimal medium. The results are given in table 1.

TABLE 1

CHARACTERISTICS OF f_1 CULTURES OBTAINED BY THE GERMINATION OF SPORES DISSECTED IN ORDER FROM WHOLE ASCI SECURED FROM CROSSES OF LEUCINELESS-ADAPTED BY LEUCINELESS

CROSS:	α × 33757-a		β × 33757-a				γ × 33757-4637-A[a]	
SPORE NO.	COLOR[b]	GROWTH[c] ON MINIMAL MEDIUM	COLOR	GROWTH ON MINIMAL MEDIUM	COLOR	GROWTH ON MINIMAL MEDIUM	COLOR	GROWTH ON MINIMAL MEDIUM
1	−	−	+	+	+	−	+	−
2	−	−	+	+	+	−	+	+
3	+	+	+	+	+	−	−	−
4	+	+	+	+	+	−	[d]	[d]
5	+	−	−	−	−	+	−	+
6	+	−	−	−	−	+	+	+
7	−	+	−	−	−	+	+	−
8	−	+	−	−	−	+	−	+

[a] This ascus was not dissected in order.

[b] + refers to salmon conidial pigment, − to albino.

[c] + refers to growth, − to failure to grow. All strains grew on medium to which leucine was added.

[d] This spore did not germinate.

In view of the 1:1 ratios obtained in these asci, leucine-independence in the adapted strains must be due to a chromosomal and not a non-Mendelian cytoplasmic factor. These crosses do not, however, supply any information about the relationship of the leucineless locus in strain 33757, l_1, to the gene for leucine-independence, L.

The demonstration of this relationship depends on crosses made between prototrophic strains from the f_1 and wild type. In performing this cross, the f_1 was used rather than the parent adapted strain to avoid the

confusion that might arise from the heterocaryotic[6] persistence of leucineless nuclei in the adapted culture. Barring mutation, a culture derived from a single ascospore is genetically homogeneous. The spores secured from these crosses are classified in table 2. The most extensive test was performed on the progeny of β where there were 13 asci in which every spore germinated, 5 asci in which at least one member of all four pairs of spores germinated so that each of the 4 chromatids could be accounted for, 66 spores from asci with incomplete germination and 83 spores isolated at random. In addition some data were secured on the progeny of crosses between wild-type and the prototrophic f_1 strains secured from α and γ. A total of 300 single-spore cultures was examined and every one proved to be prototrophic.

TABLE 2

CLASSIFICATION OF THE ORIGIN OF SINGLE-SPORE CULTURES FROM CROSSES OF PROTOTROPHIC f_1 CULTURES WITH WILD TYPE. ALL CULTURES PROVED TO BE LEUCINE-INDEPENDENT

(f_1 FROM CROSS OF)	×	WILD TYPE	NO. OF ASCI WHOSE 8 SPORES GERMINATED	NO. OF ASCI WITH ALL CHROMATIDS ACCOUNTED FOR (4–7 SPORES)	NO. OF SPORES FROM INCOMPLETE ASCI	NO. OF SPORES ISOLATED AT RANDOM
$\beta \times$ 33757-a (spore 1)		15300-A	0	3	10	0
$\beta \times$ 33757-a (spore 2)		15300-A	1	0	1	0
$\beta \times$ 33757-a (spore 3)		15300-A	12	2	47	83
$\beta \times$ 33757-a (spore 4)		15300-A	0	0	8	0
$\alpha \times$ 33757-a (spore 2)		15300-A	0	4	2	0
$\gamma \times$ 33757–4637-A (spore isolated at random)		43–14-A	0	3	7	0
			—	—	—	—
TOTAL			13	12	75	83

If leucine-independence in the adapted leucineless strains were due to mutation at a locus distinct from l_1, crosses between adapted and wild-type strains should have had in their progeny a recombination class of leucineless. In the 25 asci and among the 118 additional spores listed in table 2 there were no recombinations. Since these numbers test for 109 chances for recombination, of which none were fulfilled, the genes involved are probably alleles.

Physiological Studies.—The physiological behavior of the adapted strains confirms the hypothesis of reverse mutation. The rate of progression of the adapted strains and their prototrophic f_1 progeny on an agar surface is the same as that of wild-type strains[7] from which they were originally derived (table 3). The addition of leucine did not stimulate or retard growth in either case. After $8^1/_2$ days in 50 ml. of liquid medium containing 1 per cent sucrose, wild-type strain 1-A, albino strain 4637-A and adapted

strain β gave mycelial crops weighing between 109 and 114 mg. Thus, in their growth, as in their genetic behavior, leucineless-adapted cultures are indistinguishable from wild type. They probably represent back-mutations of the l_1 locus to the wild-type allele.

TABLE 3

RATE OF GROWTH IN MM. PER HR. OF LEUCINELESS-ADAPTED COMPARED WITH THAT OF WILD TYPE AT 25°C. EACH RATE IS THE AVERAGE OF MEASUREMENTS ON TWO GROWTH TUBES

STRAIN	MINIMAL AGAR SUPPLEMENTED WITH: 0	7.5 γ $l(+)$ LEUCINE PER ML.	2 MG. dl LEUCINE PER ML.
L-A	4.3	4.1	4.2
R977-a	4.0	...	...
4637-*A*	4.1	...	...
15300-*A*	4.4	4.2	...
α	4.2	4.4	4.3
β	4.2	4.1	4.2
Leucine-independent spore 1 from f_1 of β (see table 1)	4.2	4.2	4.3

Are the Mutations Induced?—In order to determine whether the back-mutations were induced an examination was made of the frequency of adaptation in liquid medium containing various amounts of leucine. Adaptations were identified by the arbitrary method previously described.[3] Those cultures which possessed unusually high weights, more than 3σ above the mean of the other members of a series, were classified as adapted. The data are shown in table 4. The frequency of adaptations depended not only upon the temperature but was higher in low leucine concentrations than in high leucine concentrations. Both of these differences are significant by χ^2 test. Since the dependence upon leucine concentration appears to be an example of chemically induced mutation it is important to examine closely the events that take place during the $8^1/_2$-day period.

TABLE 4

EFFECT OF LEUCINE CONCENTRATION ON THE FREQUENCY OF ADAPTATIONS OF 33757-4637-*A* DURING $8^1/_2$ DAYS IN 50-ML. LIQUID MEDIUM

TEMP: MG. $l(+)$LEUCINE	25°C. NO. OF CULTURES	25°C. NO. OF ADAPTATIONS	25°C. % ADAPTATION	30°C. NO. OF CULTURES	30°C. NO. OF ADAPTATIONS	30°C. % ADAPTATION	AV. % ADAPTATION
1.00	40	0	0	20	0	0	0
0.75	40	1	3	20	3	15	7
0.50	40	0	0	20	3	15	5
0.25	40	3	8	20	5	25	13
Av. % adaptation			3			14	

The leucine concentration expressed in table 4 is the initial concentration. As growth proceeds, the medium is depleted of leucine by the mold. When

the final weight is reached, bioassay of the medium proves the exhaustion of leucine. The medium will, at that time, support the growth of wild type *Neurospora*, or of leucineless *Neurospora* if further leucine is added. Thus, leucine alone is exhausted. The time at which this takes place depends upon the initial leucine concentration. On 0.25 mg. of *l* (+) leucine the final weight of about 7.5 mg. is attained in about 3 days. On 1.00 mg. *l* (+) leucine almost 6 days are required for the development of the final weight of 37 mg. During these times the mass of mycelium is increasing and with it, presumably, the number of nuclei in which back-mutation has a chance to occur.

In order to minimize the importance of the difference in time a long-term experiment was designed in which cultures of 33757-4637-*A* started their growth on 0.25 and 0.5 mg. *l* (+) leucine per 50 ml. at 30°C. The frequency of adaptation was observed between 6 and 45 days after inoculation, during which time all cultures were exposed to subthreshold leucine concentrations. Table 5 shows the results obtained. The same criterion was

TABLE 5

EFFECT OF LEUCINE CONCENTRATION ON THE FREQUENCY OF ADAPTATIONS OF 33757-4637-*A* BETWEEN 6 AND 45 DAYS IN 50-ML. LIQUID MEDIUM AT 30°C.

MG. *l*(+)LEUCINE	NO. OF CULTURES	NO. OF ADAPTATIONS	% ADAPTATION
0.50	43	5	12
0.25	38	16	42

used for identification of adaptations. It will be observed that even during this period there is a significantly higher frequency of adaptation in the cultures which began to grow in the presence of low leucine concentrations. Indeed, the frequency is so high that the average weight of all cultures, adapted and non-adapted, is higher (25 mg.) in the 0.25 mg. leucine series than in the 0.50 mg. leucine series (20 mg.). On the spontaneous mutation hypothesis one would expect the number of adaptations to be greater in those cultures with the higher nuclear populations, the high leucine series. We find the reverse to be true—the frequency of adaptations is an inverse function of mycelial mass.

The orthodox viewpoint is that adaptive mutations are not directed by the environment of the cell but occur in a given percentage of the population per unit time, and may be selected by the environment. We have shown that adaptation, in this instance, is a mutation phenomenon. Thus far we have assumed that when a mutation to leucine-independence takes place it will be regularly selected for in the absence of leucine and result in an adaptation. However, this is not the case. Although adaptations are the result of mutation, every mutation does not yield an adaptation as the evidence presented below will indicate.

Heterocaryons.—When a back-mutation occurs in a leucineless mycelium and the mutated nucleus multiplies a heterocaryon[6] is formed consisting of a mixture of leucineless and wild-type nuclei. The phenomenon of selection in such heterocaryons was studied by artificially preparing heterocaryons between leucineless and prototrophic strains and growing them on different concentrations of leucine. Figure 1 shows the behavior of a heterocaryon between the adapted strain β, and the leucineless strain, 33757-4637-*A*, which gave rise to it. Growth was measured on the surface of agar in growth tubes containing no leucine. The leucineless strain showed, of course, no growth. The prototrophic f_1 control grew at the

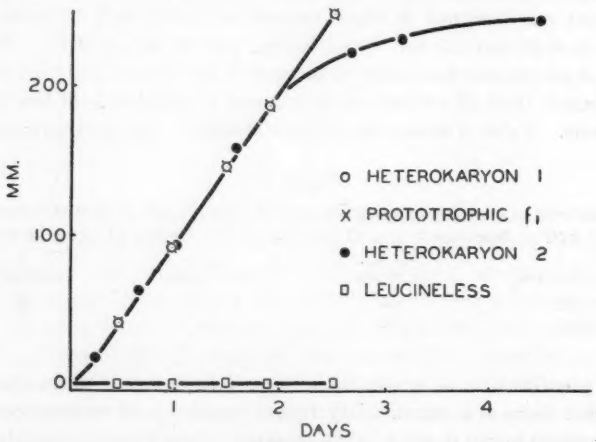

FIGURE 1

Growth rate of a heterocaryon of leucineless and adapted *Neurospora* on minimal medium.

wild-type rate of 4.2 mm./hr. The heterocaryon grew at exactly the same rate, as though the wild-type nuclei it contained had outgrown the leucineless nuclei. On medium containing a limiting concentration of leucine the prototrophic f_1 control still grew at a rate of 4.2 mm./hr. (Fig. 2). However, the heterocaryon grew at the same rate as the leucineless strain, 2.2 mm./hr. It appears as though the wild-type nuclei failed to outgrow the leucineless in the presence of a limiting leucine concentration. In other words, on minimal medium the heterocaryon grows like wild type but in the presence of leucine it grows like leucineless.

To prove that such behavior would be characteristic of hyphae known to be heterocaryotic, minimal agar plates were inoculated with a mixture of leucineless 33757-4637-*A* and of adapted strains α or β. After the mycelium had grown over an area ca. 4 cm. in diameter, tips of single hyphae

were isolated[6] and transferred to media containing or lacking leucine. The plate from which these isolations were made was also kept for further observation. Hyphae isolated to minimal medium continued to grow (as did the parent mycelium on the agar plate) demonstrating that they contained nuclei of the adapted genotype. Since hyphal tips were transferred at random, such nuclei must also have been present in those hyphae inoculated into tubes of leucine-containing medium. The conidia formed in these tubes, however, did not grow when tested on minimal medium. Therefore, in the presence of leucine, hyphae which originally contained some leucine-independent nuclei gave rise to conidia which were purely

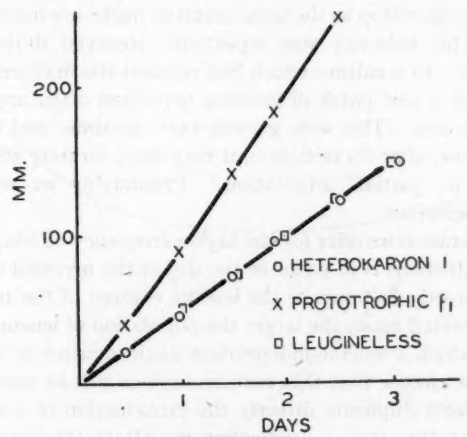

FIGURE 2

Growth rate of a heterocaryon of leucineless and adapted *Neurospora* on a limiting concentration of *l* (+) leucine (0.0075 mg./ml.).

leucineless. Such behavior was characteristic of combinations of leucineless with wild-type strains obtained independently of the leucineless mutation. The similarity of adapted and wild-type strains in this respect is further evidence for the identity of the adapted and wild-type strains at the *L* locus.

This phenomenon is under further study but the following evidence may be reported briefly here. Hyphae were isolated in a similar manner from heterocaryons in which the leucineless and wild-type nuclei bore different marker-genes for conidial color. Such heterocaryotic hyphae behaved similarly to those described above. Furthermore, whenever a leucineless culture was selected from a heterocaryon, the color markers of the wild type could not be demonstrated. This is in favor of the hypothesis that

the wild-type nuclei in such combinations were selected against in the presence of leucine. The disappearance of color markers which characterized leucineless nuclei demonstrated a selection in favor of wild type when heterocaryons were grown on minimal medium. However, in some instances when heterocaryons were studied in growth tubes on minimal medium, they grew like wild type for a time but then slowed down and sometimes stopped (Fig. 1). This behavior helps to interpret the results of the long-term growth experiment in liquid medium.

In liquid cultures when a back-mutation to leucine independence occurs and a heterocaryon is formed the independent nuclei may overgrow and form a complete adaptation. On the other hand, the heterocaryon may begin to grow and then stop as the back-mutated nuclei are inactivated by the leucineless. This behavior was repeatedly observed during growth in liquid medium. In a culture which had reached the maximum growth for its leucine level a new patch of growing mycelium often appeared on the surface of the clot. This new growth may continue and overgrow the whole culture, or, after its initiation, it may stop, forming what was previously termed a "partial" adaptation.[3] Presumably we were observing heterocaryon selection.

The interpretation we offer for the higher frequency of adaptation in low leucine concentrations is in terms of the size of the mycelial mass involved rather than directly in terms of the leucine content of the medium. The greater the mycelial mass, the larger the population of leucineless nuclei in the midst of which a leucine-independent nucleus arises by mutation and the greater the chance that this mutant nucleus will be inactivated. Although we cannot duplicate directly the introduction of a single leucine-independent nucleus into a leucineless mycelium we have studied the growth of heterocaryons in liquid medium. Figure 3 shows the weights assumed after $8^1/_2$ days by a heterocaryon of β and 33757-4637-*A* and by 33757-4637-*A* on different leucine concentrations.

As the leucine concentration rises the amount of mycelium, and hence the number of leucineless nuclei, similarly increases. However, the final weight of the heterocaryon decreases with leucine concentration. Apparently the greater the number of leucineless nuclei the more rapidly the leucine-independent nuclei are inactivated and the sooner growth ceases. These experiments, then, provide evidence for our interpretation of the frequency of adaptations on different leucine concentrations. An adaptation is due to the growth of leucine-independent nuclei which arose by mutation but whether the increased growth will be significant depends upon whether conditions favor the inactivation of the leucine-independent nuclei. Large mycelial masses with many leucineless nuclei are more unfavorable for escape of the prototrophic growth than small mycelial masses. We do not believe that our experiments allow any conclusion as to the rôle of

leucine in the induction of mutation at this locus. Such mutations probably occur spontaneously but their expression depends upon the leucine content of the environment through its effect on mycelial mass.

Discussion.—Because of the heterocaryon selection the back-mutation of locus l_1 is not a favorable object for the study of mutation rates. For example, the effect of temperature shown in table 4 may be due to its influence either on the mutation rate or on the selection efficiency or both. Any calculation of mutation rate would yield a minimum value, at best, because many, if not most, mutations can never express themselves as adaptations because of the rapid inactivation of mutant leucine-independent nuclei.

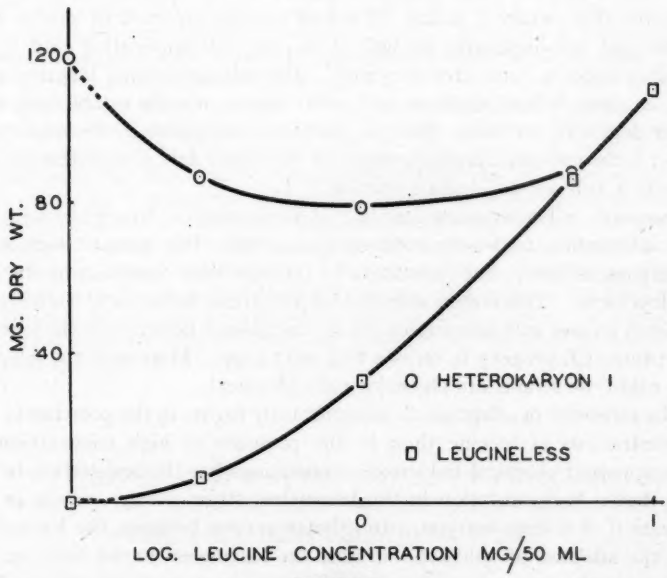

FIGURE 3

Final growth of a heterocaryon of leucineless and adapted *Neurospora* on different concentrations of *l* (+) leucine in liquid medium at 25° C.

On the basis of the selection phenomenon it is to be expected that back-mutations of locus l_1 would not persist in stock cultures maintained on leucine. This probably explains the fact that in our experience no culture in stock tubes has ever adapted. Likewise in nature the heterocaryon selection phenomenon may help to maintain mutant types.

In bacteria nutritional mutants are known to adapt and lose their requirement.[8] In this way they seem to gain a new function. In the light

of recent evidence it is conceivable that nutritional mutants in bacteria are formed by an inactivation of a gene.[9] From this state it may later back-mutate. We conceive of this process in *Neurospora* as follows:

$$L \underset{\text{back-mutation}}{\overset{\text{mutation}}{\rightleftharpoons}} l_1$$

The wild-type allele, L, is present in the wild-type stock and controls some step in the synthesis of leucine. Under the influence of ultra-violet light, or otherwise,[10] this gene can mutate to the inactive state, l_1. We conceive that, where L makes an active enzyme involved in leucine synthesis and self-duplicates as well, l_1 is also self-duplicating and could possibly make a "defective enzyme." The self-duplicating inactive gene l_1 spontaneously back-mutates to L. We believe it to be established, with a fair degree of certainty, that the leucineless mutation in *Neurospora*, l_1, is not a chromosomal rearrangement or deficiency but a modification of a gene to a still self-duplicating particle.[11]

Summary.—The leucineless mutant of *Neurospora* is a true gene mutation. The adaptation to leucine-independence, which this mutant sometimes undergoes, is due to back-mutation to the wild-type condition at the leucineless locus. This is demonstrated by the genetic behavior of the adapted strain in crosses with leucineless and by the genetic behavior of the leucine-independent f_1 progeny in crosses with wild type. Moreover, the adapted and wild-type strains are physiologically identical.

The incidence of adaptations is significantly higher in the presence of low concentrations of leucine than in the presence of high concentrations. This apparent chemical induction of mutations has its explanation in the fact that a back-mutation in the leucineless strain always results in the formation of a heterocaryon. In a heterocaryon between the leucineless and the adapted or wild-type strains, the leucineless nuclei have an advantage in the presence of leucine. Whether a back-mutation will result in an adaptation depends upon whether conditions favor selection against the leucine-independent nuclei.

[1] Beadle, G. W., *Physiol. Rev.*, **25**, 643–663 (1945); Beadle, G. W., and Tatum, E. L., *Am. Jour. Bot.*, **32**, 678–686 (1945).

[2] Regnery, D. C., *Jour. Biol. Chem.*, **154**, 151–160 (1944).

[3] Ryan, F. J., and Brand, E., *Ibid.*, **154**, 161–175 (1944).

[4] The experimental procedures used in these studies have been described previously. See references 1 and 3 and Ryan, F. J., Beadle, G. W., and Tatum, E. L., *Am. Jour. Bot.*, **30**, 784–799 (1943).

[5] We propose to designate as a prototroph any strain which has the nutritional requirements of the "wild type" from which it was derived irrespective of how it became

prototrophic. (For *Neurospora crassa* see Butler, E. T., Robbins, W. J., and Dodge, B. O., *Science*, **94**, 262 (1941).)

[6] Beadle, G. W., and Coonradt, V. L., *Genetics*, **29**, 291–308 (1944).

[7] Different "wild-type" stocks undoubtedly carry gene differences which can modify such physiological characteristics as growth rate and degree of conidiation.[4] These differences may segregate to different stocks. Therefore, it is not strictly correct to speak of the wild type as an entirely distinctive genotype. However, in every mutant studied the nutritional requirement is determined by a single gene, although the genetic background may, to a certain extent, modify the details of its expression. It would be desirable to use "isogenic" strains, obtained by repeated back-crossing, but the biparental inheritance of *Neurospora* makes very difficult the elimination of any gene differences that may be linked to mating-type alleles.

[8] Roepke, R. R., Libby, R. L., and Small, M. H., *Jour. Bact.*, **48**, 401–412 (1944).

[9] Gray, C. H., and Tatum, E. L., these PROCEEDINGS, **30**, 404–410 (1944).

[10] The leucineless mutant, 33757, was obtained from a culture of *Neurospora* which had been treated with ultra-violet radiation. However, since parallel studies on spontaneous mutation were not carried out[1] it cannot be proved that the mutation $L \rightarrow l_1$ was induced and did not occur spontaneously.

[11] Strain 4637-*A* is known to carry a translocation (McClintock, B., *Am. Jour. Bot.*, **32**, 671–678 (1945)) which is closely linked to albino. It reduces crossing over in a region of the sex chromosome (Doermann, A. H., *Arch. Biochem.*, **5**, 373–384 (1944)). The prototrophic f_1 strain used in these experiments, although wild type in color, may have carried the translocation. The adaptation could have been due to mutation to leucine independence at a locus other than l_1 since, in the cross of the f_1 with wild type, reduction of crossing over might have prevented the appearance of recombinations. There are two types of evidence which militate against these assumptions. First, we found no reason to believe that the f_1 by wild-type cross produced the lethal classes which would be expected if a translocation were involved. Second, the physiological identity of adapted strains with wild type speaks for the identity of the leucine-independent gene and the wild-type allele of l_1.

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*VARIETIES AND MATING TYPES IN PARAMECIUM BURSARIA. I. NEW VARIETY AND TYPES, FROM ENGLAND, IRELAND, AND CZECHOSLOVAKIA**

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Paramecium bursaria consists of a number of varieties (Jennings,[1] Jennings and Opitz[2]). Members of any one variety do not, as a rule, mate with members of other varieties. In each variety there is a definite number of mating types. Animals of diverse mating types conjugate readily; animals of the same mating type usually do not mate together. Variety I contains four mating types designated A, B, C, and D. Variety II has eight mating types designated E, F, G, H, J, K, L, and M. Variety

III contains four mating types designated N, O, P, and Q. Variety IV, as far as it is known, contains two mating types designated R and S. In addition to these four varieties there are certain Russian clones (denominated Ru2O to Ru35) that do not conjugate with any of the four varieties (Jennings and Opitz[2]). These workers were of the opinion that these Russian clones possibly belong to a fifth variety. In the present paper they are called the fifth variety. (Apparently only one mating type of this variety has been found, and this type is designated T.) There are known, therefore, five varieties containing nineteen mating types in this species of Paramecium.

The present paper reports the finding of a new variety (Variety VI) containing four mating types. These animals were collected from England, Ireland, and Czechoslovakia.

Material and Methods.—In July and August, 1944, cultures of two clones of *P. bursaria* were received from Professor E. G. Pringsheim in Cambridge, England. One clone (Ck1) was collected in Prague, Czechoslovakia. It was first used in his extensive experimental studies while he was in Czechoslovakia; it was later brought by him to England. This clone has been in cultivation in the laboratory for approximately twenty years. The other clone (En1) was collected in 1944 in Cambridge, England.

In June, 1945, cultures of four additional English clones (En2, En3, En4, En5) were received from Professor Pringsheim. These were collected from Newnham, near Cambridge, England.

In June, 1945, a culture of *P. bursaria* from Dublin, Ireland, was also received from Professor Pringsheim. These animals were collected by Mr. Douglas Glen of Dublin and forwarded to the writer by Professor Pringsheim. A single animal was isolated from this culture and grown in isolation. This clone was designated as Ir1.

It is a pleasure to acknowledge my indebtedness to Mr. Glen and especially to Professor Pringsheim for sending these paramecia.

The animals were cultured in essentially the manner described by Jennings.[1] For cytological studies the animals were fixed in Schaudinn's fluid containing glacial acetic acid, stained in iron hematoxylin, and destained in saturated aqueous solution of picric acid, following the technique the writer has described previously (Chen[3]).

Experimental Studies.—1. Determination of Variety and Mating Types: Two series of tests were carried out on the Czechoslovakian, Irish, and English clones: (1) to determine whether these seven clones would conjugate with each other, and (2) to determine whether these clones belong to any of the five varieties already known.

To determine whether these seven clones would conjugate with each other, mixtures were made in all possible combinations of two of the seven clones. The results of these numerous tests are shown in table 1. An

analysis of the data shows that these seven clones belong to the same variety and that four mating types are present. Clones Ck1 and Ir1 belong to one mating type; they do not mate with each other but both mate with all other five clones. Clone En1 belongs to a second mating type; it mates with all other six clones. Clone En2 belongs to a third

TABLE 1

RESULTS OF MIXING IN ALL POSSIBLE COMBINATIONS OF TWO OF THE SEVEN CLONES OF *P. bursaria* COLLECTED FROM CZECHOSLOVAKIA, IRELAND, AND ENGLAND. THE PLUS SIGNS INDICATE THAT CLUMPING AND CONJUGATION OCCUR IN THE MIXTURE OF THE TWO CLONES INDICATED; THE MINUS SIGNS THAT THEY DO NOT

	Ck1	Ir1	En1	En2	En3	En4	En5
Ck1	−	−	+	+	+	+	+
Ir1	−	−	+	+	+	+	+
En1	+	+	−	+	+	+	+
En2	+	+	+	−	+	+	+
En3	+	+	+	+	−	−	−
En4	+	+	+	+	−	−	−
En5	+	+	+	+	−	−	−

mating type; it also mates with all other six clones. Clones En3, En4, and En5 belong to a fourth mating type; they do not mate with each other but they all mate with the other four clones.

Under proper conditions, the animals of diverse mating types when mixed exhibited the agglutinative mating reaction immediately. In a few

minutes large clots or masses of animals were formed. Later these clots and masses broke up and many conjugating pairs were found. If a large number of animals of these clones were mixed, hundreds of pairs could be found in a single mixture. The behavior in mating reaction and pair formation as well as the nuclear changes during conjugation (described later in this paper) appeared to be normal and typical of this species.

Many tests were carried out to determine whether the seven clones would mate with animals belonging to any of the five already known varieties. These tests were carried out in the following manner. Cultures of many clones belonging to all of the nineteen known mating types constituting the five varieties were kept in the laboratory. These are known as "testers"; while the cultures of the Czechoslovakian, Irish, and English clones were designated "unknown." Before testing, a number of animals were taken out from one "tester" and mixed with those of another "tester" of an appropriate mating type (animals of a diverse mating type belonging to the same variety) to determine whether the "testers" were in a sexually reactive condition. The animals were considered sexually reactive if when mixed they would agglutinate immediately and form pairs. Animals of the "unknown" clones were similarly tested with each other. Tests between the "unknown" and the "testers" were carried out only when both were sexually reactive. In actual testing a number of animals of an unknown clone were mixed separately with animals from different mating type "testers." These mixtures were examined a few hours after preparation and also examined daily during the following four days to determine whether any pairs or clots were formed.

Representative clones of two of the four mating types (the Czechoslovakian clone Ck1 and the English clone En1) were tested separately with all four mating types of Variety I, seven of the eight mating types of Variety II, two of the four mating types of Variety III, the two mating types of Variety IV and the single mating type of Variety V. Altogether 77 tests were carried out on the Czechoslovakian clone, and an equal number on the English clone En1.

The results of these tests can be summarized as follows: No mating reaction or conjugation was ever found in any of the tests except in some of the cases when the English clone and a fifth variety clone (Ru22) were mixed. In such a mixture pairs could be found but most of these pairs were atypical in that the two conjugants did not have the relative positions typical of normal conjugating pairs. It was soon found out that there was really no conjugation between the English clone and the fifth variety; the conjugating pairs each consisted of two English animals, the fifth variety animals taking no part in the conjugation. (The pair formation was induced by the fluid of the fifth variety.[4]) This non-conjugation between English animals and the fifth variety was proved in the following manner:

(1) When dark green English animals were mixed with white fifth variety animals, every pair consisted of two dark green animals. (2) The individuals of the English clone are much smaller than the fifth variety animals. When the English and the fifth variety animals were mixed, each pair consisted of two small animals of similar size. (3) There are differences in the shape of the micronuclei between the English clone and the fifth variety, the latter having a much longer micronucleus. Fixed and stained pairs from a mixture of English and the fifth variety animals show that the micronuclei in two conjugants of each pair were alike; both micronuclei were short. (4) As already stated, although some of the pairs found in a mixture of English and the fifth variety animals are typical, others are atypical in that the two conjugants do not have the relative positions typical of normal conjugating pairs. If the English animals were placed in the fluid of the fifth variety, similar pairs (mostly atypical) were formed.[4]

Judging from the results of the numerous tests just described, it is obvious that these two clones do not belong to any of the five known varieties. They, together with the other five clones, clearly constitute a new variety containing at least four mating types. This new variety is to be known as the sixth variety, and the four new mating types are designated as types U, V, W, and X. Thus, there are now known in this species of Paramecium six varieties containing twenty-three mating types, which are diagrammatically presented in figure 1. The twenty-three types are designated by capital letters A–X. The small letters or figures in the lower half of the squares are designations of the clones exemplifying each mating type.

2. *Temporary Pair Formation:* With the exception of the Irish clone (Ir1) all clones of this variety are normal in that when they are mixed with clones of a different mating type, lasting pairs are formed. When the Irish clone is mixed with normal clones of a different mating type the pairs that are formed are not lasting but separate within a few hours. For example, if the Irish clone is mixed with clone En2 at about noon, strong agglutinative mating reaction occurs almost immediately. Clots of animals are formed within a few minutes. Within an hour or two, many pairs are formed but these pairs are not lasting. During the late afternoon and in the evening the pairs break up into single animals. If such a mixture is placed in a moist chamber and kept from drying, the typical agglutinative mating reaction and temporary pair formation recur the following day and daily for some successive days (perhaps for many days). The following is the characteristic daily behavior of the animals in such a mixture. The agglutinative mating reaction begins at about noon. Many pairs are formed in the early afternoon. In the late afternoon the pairs begin to break up into single animals so that by evening only a few pairs may be found; none are present by eleven o'clock P. M.

Similar phenomena are observed if the Irish clone is mixed with the following clones of the new variety: En1, En3, En4, and En5.

The temporary pair formation described in the present paper is similar to that reported by Sonneborn[5] in *P. aurelia* and by Jennings[6] and Chen[7] in *P. bursaria*.

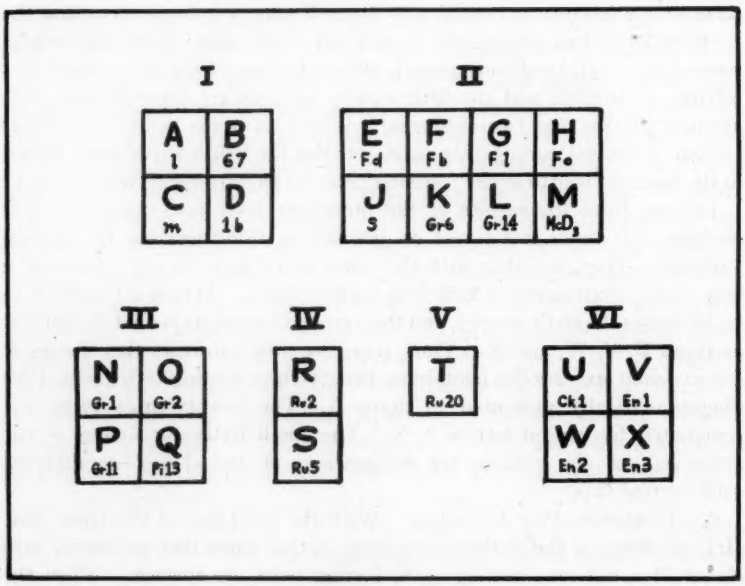


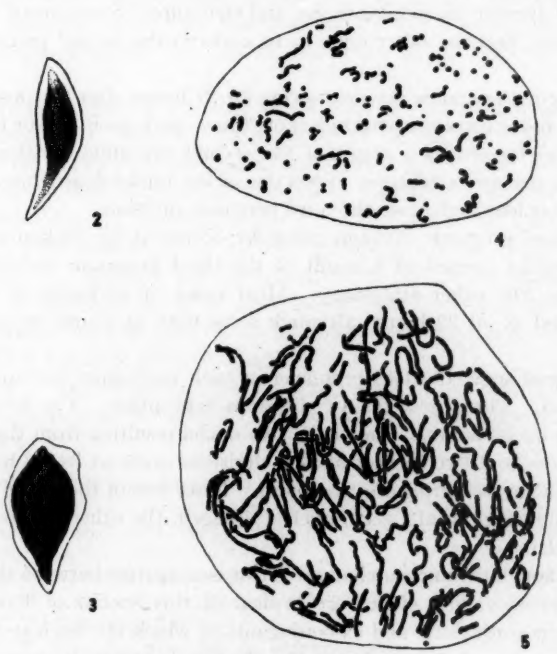
FIGURE 1

Diagram of the six known non-interbreeding varieties of *P. bursaria*. The capital letters are the designations of mating types in each variety: four in Variety I, eight in Variety II, four in Variety III, two in Variety IV, four in Variety VI. In Variety V apparently only one mating type is known. The figures or numbers in the lower half of the squares are the designations of clones that first exemplified each mating type.

Cytological Studies.—Cytological studies were carried out to determine whether nuclear changes during conjugation in this variety are normal. The Czechoslovakian clone Ck1 (mating type U) and the English clone En1 (type V) were used.

These two clones differ greatly in their micronuclei and in their chromosomes. The Czechoslovakian clone has a very small, lightly staining, ellipsoidal micronucleus (Fig. 2), whereas the English clone has a much larger and deeply staining micronucleus containing a much greater quantity

of chromatin (Fig. 3). As is seen clearly during the late prophase of the first pregamic division in conjugation, the chromosomes of the Czechoslovakian clone are for the most part either spherical or very short rods (Fig. 4), whereas most of the chromosomes of the English clone are much longer (Fig. 5). In this connection, it may be stated that this is the first variety in which I have found such great chromosomal differences between



FIGURES 2 TO 5

Figure 2, resting micronucleus of a vegetative animal belonging to clone Ck1. Figure 3, resting micronucleus of a vegetative animal belonging to clone En1. Figure 4, chromosomes of clone Ck1 as seen during the late prophase of the first pregamic division in conjugation. Figure 5, chromosomes of clone En1 as seen during the late prophase of the first pregamic division in conjugation. All figures × 1640.

clones of the same variety. In my experience, the chromosomes of clones of the same variety are similar, though marked chromosomal differences may exist between varieties. For example, Varieties II and IV are characterized by the presence of thin and short chromosomes, whereas the chromosomes in Variety III are larger and much longer.

1. Nuclear Changes during Conjugation: Nuclear changes during con-

jugation between these two clones are normal and typical of the species. They include three pregamic divisions, the exchange and fusion of pronuclei, and the three post-zygotic divisions. In the following paragraphs these nuclear changes and the time relationships of stages (at 26°C.) will be described.

The first pregamic division is a long process requiring approximately 24 hours for completion. The daughter nuclei formed as a result of the first pregamic division are similar in size and structure. Soon one of the nuclei degenerates, and the other remains to undergo the second pregamic division.

The second pregamic division occurs 25–27 hours after the onset of conjugation and is consummated in a short time—perhaps in one or two hours. The nuclei formed as a result of the second pregamic division are also similar in size and structure. Soon one of the nuclei degenerates; the remaining nucleus undergoes the third pregamic division.

The third pregamic division generally occurs at 28–30 hours. Of the two pronuclei formed as a result of the third pregamic division, one is migratory, the other stationary. Most cases of exchange of pronuclei were found at 30–32 hours, although some may be found as early as 29 hours.

After exchange, the two pronuclei in each conjugant fuse and form a synkaryon. Three post-zygotic divisions take place. The first division occurs at 32–33 hours. One of the two nuclei resulting from this division degenerates, and two further nuclear divisions occur at 34–38 hours. By the 38th hour, ex-conjugants are found. Later two of the nuclei in the ex-conjugants develop into macronuclear anlagen, the other two become the micronuclei.

Even though the nuclear changes during conjugation between the English and Czechoslovakian clones are typical of this species of Paramecium, there occur conjugants and ex-conjugants in which the nuclear conditions are abnormal, and the number of the abnormal animals is unusually high. Some of the abnormal nuclear conditions can be listed: (1) There are a number of conjugants (mostly Czechoslovakian conjugants) in which both of the nuclei resulting from the first pregamic division persist and undergo the second pregamic division. (2) There are many conjugants in which the pronuclei, after exchange, do not fuse together to form a synkaryon but each pronucleus develops into a hemikaryon.[8] (3) The number of nuclei in the ex-conjugants is unusually variable. In this species of Paramecium the normal number of nuclei in the ex-conjugants immediately after separation is four (two of these later develop into macronuclear anlagen, the other two become the micronuclei). Among the ex-conjugants resulting from conjugation between the English and Czechoslovakian clones approximately fifty per cent of the animals have the normal number of nuclei;

in the other fifty per cent the number of nuclei varies from two to twenty. It is difficult to explain the unusually high percentage of abnormal conjugants and ex-conjugants. Possibly it is due to some degree of incompatibility between these two clones or to the fact that one of these two clones (Ck1) is a very old clone, having been cultured in the laboratory for approximately twenty years. The English clone at the time of mating with the Czechoslovakian clone was about a year old. Jennings[9] found that in *P. bursaria* when an old clone conjugates with a young one, most or all of the ex-conjugants die (just as when two old clones conjugate together).

2. Differences between Varieties in the Rate of Nuclear Changes during Conjugation: In *P. bursaria* there appear to be marked differences between certain varieties in the rate of nuclear changes during conjugation. For example, under comparable conditions the rate of nuclear changes during conjugation in Variety I is much faster than that in Variety II (at least in the cases studied). At 26.5°C. all of the nuclear changes during conjugation in Variety I are completed in 20–22 hours, whereas those in Variety II continue for 36 or more hours. In Variety I the first pregamic division requires but 12 or 13 hours for completion, whereas in Variety II it requires 20 or 21 hours.

The rate of nuclear changes during conjugation in the new variety (VI) is comparable to that of Variety II; it is certainly much slower than that of Variety I.

* This work has been aided by grants from the Committee for Research in Problems of Sex, National Research Council; from the Joseph Henry Fund of the National Academy of Sciences; and from the Permanent Science Fund of the American Academy of Arts and Sciences.

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ON BLOCKS OF CHARACTERS OF GROUPS OF FINITE ORDER, I

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1. The present paper is a continuation of an earlier investigation.[1] Let G be a group of finite order g, and let $\Gamma = \Gamma(G)$ denote the corresponding group ring formed with regard to an algebraic number field K. We shall assume that all the simple constituents of the semisimple ring Γ split completely in K. This hypothesis holds, for example, when K contains the gth roots of unity.[2] Let p be a rational prime number and let $\mathfrak{p}$ be a prime ideal of K dividing p. The ordinary irreducible characters ζ_μ of G and the modular characters φ_ν of G (for p) are distributed into a certain number of "blocks"[3] $B_1, B_2, \ldots, B_t$, each ζ_μ and each φ_ν belonging to exactly one block B_τ. As was mentioned in A G R, these blocks are linked closely with the arithmetic in Γ.

We are interested in obtaining relations between the blocks of G and those of certain subgroups N. These N will be the normalizers of the p-subgroups of G (and some related groups). This will mean that a number of important features of the characters of G are determined by the structure of these groups N and the position of N in G, in particular, the manner in which the classes of conjugate elements of N are distributed in the classes of G.

2. Denote the center of the group ring $\Gamma = \Gamma(G)$ by $\Lambda = \Lambda(G)$. As is well known, a basis of Λ is formed by the classes of conjugate elements $K_1, K_2, \ldots K_k$ of G, each class K_i being interpreted as the sum of all its elements. We then have formulae

$$K_\alpha K_\beta = \sum a_{\alpha\beta\gamma} K_\gamma \tag{1}$$

where the $a_{\alpha\beta\gamma}$ are rational integers, $a_{\alpha\beta\gamma} \geqq 0$.

Let H be any subgroup of G of an order p^h, $h \geqq 0$, where p is the fixed prime selected above. Denote by $\mathfrak{C}(H)$ the centralizer of H in G and by $\mathfrak{N}(H)$ the normalizer of H in G, and consider a subgroup N which satisfies the condition

$$H\mathfrak{C}(H) \subseteq N \subseteq \mathfrak{N}(H). \tag{2}$$

If K_α^0 is the part of K_α which lies in $\mathfrak{C}(H)$, then either $K_\alpha^0 = 0$ if K does not contain any elements of $\mathfrak{C}(H)$, or K_α^0 is a sum of complete classes of N. It can be shown easily that (1) implies

$$K_\alpha^0 K_\beta^0 \equiv \sum_\gamma a_{\alpha\beta\gamma} K_\gamma^0 \pmod{p}. \tag{3}$$

Consequently, the classes K_α with $K_\alpha^0 = 0$ form the basis of an ideal T^*

of the center Λ^* of the modular group ring Γ^*.[4] On the other hand, the $K_\alpha^0 \neq 0$ can be considered as the basis of a subring R^* of the center $\Lambda^*(N)$ of the modular group ring $\Gamma^*(N)$ of N. Now (3) yields

$$R^* \simeq \Lambda^*(G)/T^*. \tag{4}$$

This relation represents a connection between the group rings of G and of N; it forms the basis of our work.

3. The algebra $\Lambda^*(N)$ is commutative and splits completely, its irreducible characters $\tilde{\omega}^*$ are all linear. The character $\tilde{\omega}^*$ of $\Lambda^*(N)$ induces a character of the subring R^*. Because of (4), this character may be interpreted as a character of $\Lambda^*(G)/T^*$ and hence it induces a character ω^* of $\Lambda^*(G)$ which vanishes for the elements of T^*. If we know how the classes of N are distributed among the classes of G, we can express ω^* explicitly in terms of $\tilde{\omega}^*$. We have

$$\omega^*(K_\alpha) \equiv \sum \tilde{\omega}^*(\tilde{K}_\rho) \pmod{\mathfrak{p}} \tag{5}$$

where $\tilde{K}_\rho$ ranges over all classes of N which belong to K_α.

Every ordinary character ζ_μ of G determines a character ω_μ of $\Lambda(G)$ which is given by

$$\omega_\mu(K_\alpha) = g\zeta_\mu(\sigma_\alpha)/n_\alpha z_\mu \tag{6}$$

where σ_α is an element in the class K_α, n_α is the order of the normalizer of σ_α, and z_μ is the degree of ζ_μ. The modular characters ω^* of $\Lambda^*(G)$ are obtained by considering the different $\omega_\mu \pmod{\mathfrak{p}}$. In particular, two characters ζ_μ and ζ_ν belong to the same block B_τ, if they yield the same ω^*.

If $\tilde{B}_\sigma$ is a block of characters of N, there is associated a modular character $\tilde{\omega}^*$ of $\Lambda^*(N)$ with $\tilde{B}_\sigma$. As described above, this character $\tilde{\omega}^*$ determines a character ω^* of $\Lambda(G)$. Again, this character ω^* determines a block B_τ of G. We shall say that B_τ *is the block of* G *determined by the block* $\tilde{B}_\sigma$ *of* N. It follows from the results of A G R that the defect $\tilde{d}_\sigma$ of $\tilde{B}_\sigma$ and the defect d_τ of B_τ satisfy the inequality

$$h \leqq \tilde{d}_\sigma \leqq d_\tau \tag{7}$$

where p^h is the order of H.

4. In (2), the group N was left arbitrary to some extent. Choose N now as the normalizer $\mathfrak{N}(H)$ of H. It was shown in A G R that for a given block B_τ of G, there exist subgroups H and blocks $\tilde{B}_\sigma$ of $\mathfrak{N}(H)$ for which the equality sign holds in (7). If we consider conjugate subgroups of G as not essentially different, then H is uniquely determined. We call this group H the *defect group* H_τ of B_τ; its order is p^h with $h = d_\tau$. Again, the block $\tilde{B}_\sigma$ of $\mathfrak{N}(H_\tau)$ is uniquely determined.

Returning to the case of an arbitrary N in (2), we state

THEOREM 1: *Let H be a subgroup of order p^h of G, let N be a subgroup of G satisfying $H \cdot \mathfrak{C}(H) \subseteq N \subseteq \mathfrak{N}(H)$. If the block $\tilde{B}_\sigma$ of N with the defect group $\tilde{H}_\sigma$ determines the block B_τ of G with the defect group H_τ, then $H \subseteq \tilde{H}_\sigma \subseteq N$, and $\tilde{H}_\sigma$ is conjugate in G to a subgroup of H_τ.*

5. The k linear characters ω_i corresponding to the k irreducible characters ζ_i of G can be arranged in form of a matrix $\Omega = (\omega_i(K_j))$ of degree k. If the block B_τ contains x_τ ordinary characters ζ_i, then x_τ rows of Ω correspond to B_τ. Choose a minor Δ_τ of degree x_τ containing these x_τ rows such that Δ_τ is divisible by $\mathfrak{p}$ to the least possible power. It can then be shown that it is possible to make this selection of x_τ columns for each block B_τ in such a manner that every column appears for one and only one block. This result is by no means trivial; for the proof, the theory of algebras and the significance of blocks must be used.

Since the columns of Ω correspond to the classes K_j of G, we have associated x_τ classes K_j with every block B_τ such that every class is associated with one and only one block. The selection of classes for the different blocks may be possible in more than one way. In any case, the number of *p-regular* classes among the classes associated with B_τ can be shown to be equal to the number y_τ of modular characters in B_τ, and further these y_τ p-regular classes associated with B_τ form a selection in the sense of A G R, § 3, in particular Theorem 2.

So far we assumed that $B_1, B_2, \ldots, B_t$ were the blocks of ordinary and modular characters of G. It will be important to note that the results of this section remain valid if every B_τ is a collection of ordinary and modular characters of G, such that every ordinary and modular character of G belongs to exactly one B_τ, and that every B_τ consists of one or several blocks of G. Again x_τ denotes the number of ordinary characters and y_τ the number of modular characters in B_τ.

6. We shall say that a group H of order p^h is the *defect group* of a class K_j, if H is a p-Sylow-subgroup of the normalizer of suitable elements of K_j. This implies that p^h is the highest power of p dividing n_j in (6); the exponent h will be termed the *defect* of K_j. We can now state the following results

THEOREM 2: *Let $(\mathfrak{H})$ be a system of subgroups H of orders $1, p, p^2, \ldots$, of G such that every subgroup of order p^h of G is conjugate to exactly one H in $(\mathfrak{H})$. For every H in $(\mathfrak{H})$, find the collection $\tilde{B}^{(\tau)}$ of all blocks $\tilde{B}_\sigma$ of $\mathfrak{N}(H)$ which determine a given block B_τ of G, and select a full system of classes $\tilde{K}_\rho$ of $\mathfrak{N}(H)$, which are associated with $\tilde{B}^{(\tau)}$. Suppose that $r_\tau(H)$ of these classes $\tilde{K}_\rho$ have H as their defect group. Different ones of these $r_\tau(H)$ classes $\tilde{K}_\rho$ belong to different classes K_α of G; the classes K_α thus obtained for the different H in $(\mathfrak{H})$ form a possible selection of classes associated with B.*

As corollaries, we have

THEOREM 3: *The number of characters in B_τ is given by*

$$x_\tau = \sum_H r_\tau(H) \tag{8}$$

where the sum extends over all H in $(\mathfrak{H})$.

THEOREM 4: *If $s_\tau(H)$ of the $r_\tau(H)$ classes $\tilde{K}_\rho$ in Theorem 2 are p-regular, then the number of modular characters in B_τ is given by*

$$y_\tau = \sum_H s_\tau(H) \tag{9}$$

where H again ranges over all groups of $(\mathfrak{H})$.

THEOREM 5: *If, in (9), H ranges only over those groups of $(\mathfrak{H})$ which have a fixed order p^h, the corresponding sum*

$$y_\tau^{(h)} = \sum s_\tau(H), \ (H \text{ in } (\mathfrak{H}); \ (H:1) = p^h) \tag{10}$$

represents the multiplicity of p^h as an elementary divisor of the Cartan matrix C_τ of the block B_τ.

It would be conceivable that the numbers $r_\tau(H)$ and $s_\tau(H)$ depend on the special selection of classes of $\mathfrak{N}(H)$ associated with B_τ. However, this is not so; we have

THEOREM 6: *The numbers $r_\tau(H)$ and $s_\tau(H)$ in the preceding theorems depend only on the group G, the subgroup H and the block B_τ of G.*

7. In order to discuss our results, let us assume for the sake of simplicity that G does not contain any normal subgroup of an order $p^h > 1$. Suppose we know: (*a*) A complete system of subgroups H of a p-Sylow-subgroup of G, (*b*) which of the groups H in (*a*) are conjugate in G; (*c*) the characters of the normalizers $\mathfrak{N}(H)$, $H \neq 1$, (*d*) the manner in which the classes of conjugate elements of $\mathfrak{N}(H)$ appear in the classes of conjugate elements of G.

If $H \neq 1$, then, under our present assumption, $\mathfrak{N}(H)$ is a proper subgroup of G. If we know the characters, we can find the modular characters $\tilde{\omega}^*$ of the center $\Lambda^*(\mathfrak{N}(H))$ of the group ring of $\mathfrak{N}(H)$, and this gives us the blocks $\tilde{B}_\sigma$ of $\mathfrak{N}(H)$. Then (5) gives the modular characters ω^* of $\Lambda^*(G)$. In this manner, all the characters ω^* belonging to the different blocks B_τ of positive defect are obtained. Further, we can determine which $\tilde{B}_\sigma$ for a fixed $H \neq 1$ belong to $\tilde{B}^{(\tau)}$, and then find $r_\tau(H)$ and $s_\tau(H)$. This is not sufficient to determine x_τ and y_τ completely, since the numbers $r_\tau(1)$ and $s_\tau(1)$ remain undetermined. However, we obtain lower bounds for x_τ and y_τ. Further, since any p-singular class K_α has a positive defect, we have $r_\tau(1) = s_\tau(1)$, and hence the excess $x_\tau - y_\tau$ of the number x_τ of ordinary characters over the number y_τ of modular characters in B_τ can be obtained. Finally (10) gives the multiplicity of the elementary divisors different from 1 of C_τ. This shows that a number of the most important

invariants of the blocks are determined by the information contained in (*a*), (*b*), (*c*), (*d*).

8. It had been shown in A G R, that if for a subgroup H of order p^h in G, the group $\mathfrak{N}(H)$ contains $q(H)$ blocks of defect h, then G possesses

$$\sum_H q(H), \ (H \text{ in } (\mathfrak{H}), \ (H:1) = p^h) \tag{11}$$

blocks of defect h. It may be remarked that the number $q(H)$ can be determined by means of the group $\mathfrak{N}(H)/N = U$ and its normal subgroup $H\mathfrak{C}(H)/H = V$. The characters θ of V are distributed in classes of characters which are associated with regard to U; two characters θ and θ_1 being *associated* if

$$\theta_1(\sigma) = \theta(u^{-1}\sigma u)$$

where σ is a variable element of V and u is a fixed element of U. Then it can be shown that $q(H)$ is equal to the number of classes of associated characters θ of V of defect 0, such that no element u of U exists of order p with regard to the subgroup V for which $\theta(u^{-1}\sigma u) = \theta(\sigma)$. If $h > 0$, this result requires only the investigation of groups of smaller order than g, in order to obtain $q(H)$ and (11).

9. There does not seem to exist a similar result in the case of blocks of defect 0. As a substitute, we have here the theorem:

THEOREM 7: *The classes of defect 0 in G form the basis of a subalgebra M of the center Λ^* of the modular group ring Γ^* of G. The number of blocks of defect 0 is equal to the rank of M^* for sufficiently large n.*

* Part of the work on this and a following note was done while the author was a Fellow of the John Simon Guggenheim Memorial Foundation.

[1] "On the Arithmetic in a Group Ring," these PROCEEDINGS, **30**, 109–114 (1944). This paper will be quoted as A G R.

[2] Brauer, R., *Am. Jour. Math.*, **67**, 461–471 (1945).

[3] See for instance, Brauer, R., and Nesbitt, C., *Ann. Math.*, **42**, 556–590 (1941).

[4] We denote the residue class field of the integers of K (mod $\mathfrak{p}$) by K^* and the group ring of G with regard to K^* by Γ^*.

THE RATE OF GROWTH OF ANALYTIC FUNCTIONS

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This note presents a theorem of Phragmén-Lindelöf type and indicates how it can be applied to establish and generalize results of N. Levinson[1] on the determination of the rate of growth of an analytic function along a line

from its growth on a sequence of points. Detailed proofs will be published elsewhere.

THEOREM 1. *Let $\delta(r)$ be a continuous function such that $0 \leq \delta(r) < 1/2$ and $\int^{\infty} r^{-1}\delta(r)dr$ diverges. Let $H(z)$ be analytic in $x \geq 0$ and let it satisfy*

$$\log |H(re^{i\theta})| = o\{\omega(r)\}, \quad r \to \infty, \quad \omega(r) = \exp\{\int_n^r s^{-1}\delta(s)ds\}.$$

Let $H(z)$ be bounded on the curve $x = r\delta(r)$. Then $H(z)$ is bounded in $x \geq r\delta(r)$.

The proof depends on the following lemma.

LEMMA. *Let $u + iv = f(z)$ map the half plane $x \geq 0$ on the region $u \geq r\delta(r)$, $r^2 = u^2 + v^2$. Then for sufficiently large ρ,*

$$\max_{|\theta| \leq \pi/2} |f(\rho e^{i\theta})| \leq \eta(\rho),$$

where $s = \eta(\rho)$ is the inverse of $\rho = C\omega(s)$, C being an appropriate constant.

The lemma is a consequence of a theorem of Ahlfors on conformal mapping.[2, 3]

To prove the theorem, consider $\psi(z) = H(f(z))$. Then $H(z)$ is analytic in $x \geq 0$ and bounded on the imaginary axis, and

$$\log |\psi(re^{i\theta})| = o\{\omega(|f(re^{i\theta})|)\}.$$

By the lemma we have $|f(re^{i\theta})| \leq \eta(r)$ for large r. Since $\omega(r)$ is a non-decreasing function, $\log |\psi(re^{i\theta})| = o\{\omega(\eta(r))\} = o(r)$. By a well-known theorem of Phragmén and Lindelöf, this implies that $\psi(z)$ is bounded in $x \geq 0$, and the theorem follows.

Now let $\varphi(z)$ be analytic in $x \geq 0$ and of exponential type there. Consider the problem of finding conditions under which

$$\limsup_{x \to \infty} x^{-1} \log |\varphi(x)| = \limsup_{x \to \infty} \lambda_n^{-1} \log |\varphi(\lambda_n)|, \tag{1}$$

where, for simplicity, we suppose here that the λ_n form a real increasing sequence and $\lambda_{n+1} - \lambda_n \geq d > 0$. We suppose that $\{\lambda_n\}$ has a density, that is, that $\lim_{n \to \infty} n/\lambda_n = D, 0 \leq D < \infty$, or equivalently that $\lim_{t \to \infty} t^{-1}\Lambda(t) \geq D$, where $\Lambda(t)$ denotes the number of λ_n not exceeding t. It was shown by V. Bernstein that (1) is true if

$$\limsup_{|y| \to \infty} |y|^{-1} \log |\varphi(iy)| = \pi L, \tag{2}$$

with $D > L$. Levinson[1] gave a simpler proof and gave sharper results for the case where (2) is strengthened and $D = L$. By the use of Theorem 1 we can extend Levinson's proof of Bernstein's theorem to prove not only Levinson's sharper theorems, but also more general results, including cases

where the hypothesis that $\varphi(z)$ is of exponential type is considerably relaxed.

We first observe that, by replacing $\varphi(z)$ by $e^{-\alpha}\varphi(z)$, we may suppose that $\limsup \lambda_n^{-1} \log |\varphi(\lambda_n)| = -\epsilon < 0$. Let $F(z)$ denote the canonical product with zeros $\pm \lambda_n$. With Levinson, we consider

$$g(z) = \sum_{n=1}^{\infty} \frac{\varphi(\lambda_n)F(z)}{(z-\lambda_n)F'(\lambda_n)}, \qquad H(z) = \frac{\varphi(z) - g(z)}{F(z)}.$$

From known lemmas on $F(z)$, it follows that $H(z)$ is analytic and of exponential type in $x \geq 0$. By imposing appropriate conditions on $\{\lambda_n\}$ and on $\varphi(iy)$, the latter being a strengthened form of (2), we shall have

$$|H(re^{i\theta})| \leq A \exp \{\pi L \cos \theta - r\delta(r)\} + B$$

for $|\theta|$ near $\pi/2$, where A and B are constants and $\delta(r)$ satisfies the conditions of Theorem 1. Theorem 1 then shows that $H(x)$ is bounded, and so

$$|\varphi(x)| \leq A|F(x)| + |g(x)|,$$
$$\limsup_{x\to\infty} x^{-1} \log |\varphi(x)| \leq 0,$$

and (1) will follow.

[1] Levinson, N., *Gap and Density Theorems*, New York, 1940, chap. 7.

[2] Nevanlinna, R., *Eindeutige Analytische Funktionen*, Berlin, 1936.

[3] A similar application of Ahlfors' lemma has been made by W. H. J. Fuchs, On the closure of $\{e^{-t}t^{\alpha}{}_{r}\}$, *Proc. Cambridge Philos. Soc.*, **42**, 91–105 (1946).

A GENERALIZATION OF THE HOPF INVARIANT

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In this note we define a homomorphism of the nth homotopy group of the r-sphere S^r into the nth homotopy group of S^{2r-1} for $n < 3r - 3$. This homomorphism may be regarded as a generalization of the invariant defined by H. Hopf.[1] This homomorphism is used to construct new essential maps of spheres on spheres.

1. Let A and B be arcwise connected spaces, a_0 and b_0 points of A and B, respectively. Let $A \vee B$ be the subset $a_0 \times B \cup A \times b_0$ of the product $A \times B$; $A \vee B$ may be regarded as the union of the two spaces A and B with just one point in common. The homotopy groups of $A \times B$ and $A \vee B$ are related by

$$\pi_r(A \vee B) \approx \pi_r(A \times B) + \pi_{r+1}(A \times B, A \vee B) \quad \text{(direct sum)} \quad (1)$$

More precisely, in the homotopy sequence

$$\ldots \to \pi_{r+1}(A \times B, A \vee B) \xrightarrow{\lambda} \pi_r(A \vee B) \xrightarrow{\mu} \pi_r(A \times B) \to \ldots$$

μ is a homomorphism *onto* and λ an isomorphism into, while $\pi_r(A \vee B)$ decomposes into the direct sum of the kernel of μ (= the image of λ), with a subgroup mapped isomorphically by μ.

If A and B are spheres S^{p_1} and S^{p_2}, further results can be obtained. Let h_i be a map of the p_i-cell E^{p_i} which is topological on $E^{p_i} - \dot{E}^{p_i}$ and maps $\dot{E}^{p_i}$ into a point; and let h be the map of $E^{p_1+p_2} = E^{p_1} \times E^{p_2}$ on $S^{p_1} \times S^{p_2}$ defined by $h(x_1, x_2) = (h_1[x_1], h_2[x_2])$. Since h maps $S^{p_1+p_2-1} = \dot{E}^{p_1+p_2}$ on $S^{p_1} \vee S^{p_2}$, it induces a homomorphism $h^*: \pi_{n+1}(E^{p_1+p_2}, S^{p_1+p_2-1}) \to \pi_{n+1}(S^{p_1} \times S^{p_2}, S^{p_1} \vee S^{p_2})$. By methods similar to those of Freudenthal[2] we can prove:

(1) h^* is onto if $n < p_1 + p_2 + \min(p_1, p_2) - 2$;
(2) h^* is an isomorphism if $n < p_1 + p_2 + \min(p_1, p_2) - 3$.

It follows from (1) and known theorems that

$$\pi_n(S^{p_1} \vee S^{p_2}) \approx \pi_n(S^{p_1}) + \pi_n(S^{p_2}) + \pi_n(S^{p_1+p_2-1}) \; (n < p_1 + p_2 + \min(p_1, p_2) - 3).$$

This generalizes a result of J. H. C. Whitehead.[3]

2. Let S^{r-1} be an equator of S^r; identification of the points of S^{r-1} defines a mapping ϕ: $S^r \to S^r_1 \vee S^r_2$. Then ϕ induces a homomorphism ϕ^*: $\pi_n(S^r_1) \to \pi_n(S^r_1 \vee S^r_2)$. If $n < 3r - 3$ let ψ be the projection of $\pi_n(S^r_1 \vee S^r_2)$ on its direct summand $\pi_n(S^{2r-1})$; then $H = \psi\phi^*$ is a homomorphism of $\pi_n(S^r)$ into $\pi_n(S^{2r-1})$.

Let f, g be mappings of S^p into S^q, S^r into S^s, respectively. Represent S^{p+q+1} as the join of S^p with S^q and S^{r+s+1} as the join of S^r and S^s. Let $f * g$ be the mapping of S^{p+q+1} into S^{r+s+1} which sends the segment $\overline{xy}$ ($x \in S^p$, $y \in S^q$) linearly into the segment $\overline{f(x)g(y)}$. The element of $\pi_{p+q+1}(S^{r+s+1})$ represented by $f * g$ depends only on the elements $\alpha \in \pi_p(S^q)$, $\beta \in \pi_r(S^s)$ represented by f and g; call it $\alpha * \beta$. If $q = s$, $g =$ the identity map, then $\alpha * \beta$ is the $(s+1)$-fold Einhängung[2] of α.

Let $f: S^{p-1} \times S^{q-1} \to S^{r-1}$ be a mapping of type (α, β)[1]. Represent S^{p+q-1} as the join of S^{p-1} with S^{q-1}; and let f^* be the mapping of S^{p+q-1} into S^r which maps the segment $\overline{xy}$ ($x \in S^{p-1}$, $y \in S^{q-1}$) linearly on the great semicircle from the north pole to the south pole of S^r which passes through the point $f(x, y)$. Then if γ is the element of $\pi_{p+q-1}(S^r)$ represented by f^* and if $p + q < 3r - 2$, then $H(\gamma) = \alpha * \beta$. It follows from a result of S. Eilenberg[4] that if α is an element of $\pi_{2r-1}(S^r)$ with Hopf invariant[1] h, then

$H(\alpha)$ is h times a generator of $\pi_{2r-1}(S^{2r-1})$. Thus H may be regarded as a generalization of the Hopf invariant.

Let R_{r-1} be the rotation group of S^{r-1}, π the mapping of R_{r-1} into S^{r-1} which sends each rotation r into the image under r of a fixed point $y_0 \in S^{r-1}$. If $p < 2r - 2$ and f is a mapping of S^{p-1} into R_{r-1}, then f defines a mapping $F: S^{p-1} \times S^{r-1} \to S^{r-1}$ of type $(\pi\alpha, 1)$, where α is the element of $\pi_{p-1}(R_{r-1})$ represented by f and 1 is the element of $\pi_{r-1}(S^{r-1})$ represented by the identity map. Then F determines an element γ of $\pi_{p+r-1}(S^r)$ as in the preceding paragraph, with $H(\gamma) =$ the r-fold Einhängung $E\pi\alpha$ of $\pi\alpha$. Since $p < 2r - 2$, E is an isomorphism[2] and it follows that if $\pi\alpha \neq 0$, then $\gamma \neq 0$. This result can be used to construct essential maps of S^n into S^r with $n = 12, 14, 8k$ and $16k + 2$, and $r = 6, 7, 4k$ and $8k$, respectively.

If $r = 2, 4, 8$, Hurewicz and Steenrod[5] have proved that $\pi_n(S^r)$ is isomorphic with the direct sum $\pi_n(S^{2r-1}) + \pi_{n-1}(S^{r-1})$. This isomorphism determines a homomorphism H' of $\pi_n(S^r)$ into $\pi_n(S^{2r-1})$. It is then easy to see that if $n < 3r - 3$, then $H' = H$.

3. Let X be an arcwise connected space, f a map of S^n into S^r, and g a map of S^r into X. The correspondence $(f, g) \to gf$ defines an operation associating with $\alpha \in \pi_n(S^r)$, $\beta \in \pi_r(X)$ an element $\beta \cdot \alpha \in \pi_n(X)$. It is known that the left distributive law $\beta \cdot (\alpha_1 + \alpha_2) = \beta \cdot \alpha_1 + \beta \cdot \alpha_2$ holds, while the corresponding right distributive law is in general false. Using the homomorphism H defined above, we can prove: if $n < 3r - 3$, then

$$(\beta_1 + \beta_2) \cdot \alpha = \beta_1 \cdot \alpha + \beta_2 \cdot \alpha + [\beta_1, \beta_2] \cdot H(\alpha)$$

where $[\beta_1, \beta_2]$ is the product defined by J. H. C. Whitehead.[3]

[1] *Math. Ann.*, **104**, 637–665 (1931); *Fund. Math.*, **25**, 427–440 (1935).
[2] *Comp. Math.*, **5**, 299–314 (1937).
[3] *Ann. Math.*, **42**, 409–428 (1941).
[4] *Ibid.*, **41**, 662–673 (1940).
[5] These PROCEEDINGS, **27**, 60–64 (1941).

ON THE STABILITY OF SYSTEMS OF DIFFERENTIAL EQUATIONS

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1. Consider the system of differential equations over the interval $0 \leq t \leq \infty$

$$\frac{dx_i}{dt} = \sum_{j=1}^{n} a_{ij}x_j, \; i = 1, \ldots n \tag{1}$$

and the perturbed system

$$\frac{dy_i}{dt} = \sum_{j=1}^{n} a_{ij}y_j + f_i(y_1, \ldots y_n, t). \tag{2}$$

We are interested in determining conditions on the f_i and $a_{ij}(t)$ which will ensure that the solutions of (2) have properties similar to those of (1). The classical stability theory of Liapounoff,[4] where (1) is regarded as an approximation to (2), for particular f_i, is included in questions of this sort, as is also some fairly recent work of Cesari,[3] treating the case where the f_i are linear in the y_k. For the case of linear perturbation, a simple method of proof, sufficient also for the case of variable a_{ij}, was obtained by the author,[1] for the particular case of nth order linear differential equations. The same methods apply to linear systems.

Here, however, we are interested in a more general situation, and the purpose of this note is to indicate how Liapounoff's[4] and Cesari's[3] investigations are special cases of one more general result, which can be proved simply and directly by use of a classical method, the Picard iteration process.

For simplicity, we write the equation in vector form. Let x be the column vector with components x_i, $i = 1, \ldots n$, $f(x, t)$ the column vector whose components are $f_i(x_1, \ldots x_n, t)$, $i = 1, \ldots n$, and let A be the matrix a_{ij}, $i, j = 1, \ldots n$.

Equation (1) becomes

$$\frac{dx}{dt} = Ax \tag{3}$$

and (2) becomes

$$\frac{dy}{dt} = Ay + f(y,t). \tag{4}$$

Furthermore, define

$$\|x\| = (\sum_i |x_i|^2)^{1/2}. \tag{5}$$

If $\|x\|$ is bounded as $t \to \infty$, we say that the solution is bounded. The following results can now be stated:

THEOREM 1: *If all the solutions of (3) are bounded, all the solutions of (4) are bounded, provided:*

(1) A is a constant matrix.

(2) $\|f(y,t)\| \leq c_m\phi(t)$ for $\|y\| \leq m$, and

(3) $\int^{\infty} \phi(t)dt < \infty$.
(4) $\|f(y,t) - f(z,t)\| \leq d_m\psi(t)\|y - z\|$ for $\|y\| \leq m$, and
(5) $\int^{\infty} \psi(t)dt < \infty$.

THEOREM 2: *The same result holds if (1) is replaced by (1)':*

$$(1)' \quad \left|\int_0^t (tr\, A)dt\right| \leq c < \infty, \qquad 0 \leq t \leq \infty. \tag{6}$$

Conditions (2) and (3) are satisfied if $f(y, t)$ has all components of the form $g(t)h(y)$, where

$$\int^{\infty} g(t)dt < \infty \tag{7}$$

and $h(y)$ has a continuous derivative with respect to y for $0 \leq y < \infty$.

Condition (3) is added to ensure that all solutions of the perturbed system are bounded. If it is a question of exhibiting perturbed solutions which are close to the original solution, the following result may be applied:

THEOREM 3: *If all solutions of (3) are bounded, there exist bounded solutions of (4), provided:*

(1) A is a constant matrix.
(2) $f(y, t)$ is a continuous function of y, and
(3) $\|f(y,t)\| \leq c_m\phi(t)$, $\int^{\infty} \phi(t)dt < \infty$.

Furthermore, y can be chosen to have the same value as x at a point, t_0, and for $t \geq t_0(\epsilon)$, $\|y - x\| \leq \epsilon$.

As in Theorem 2, this result can be extended to variable A.

If a stronger requirement is imposed upon the matrix A, a more liberal perturbation is allowed. Thus, if we insist that the characteristic roots of

$$|A - \lambda I| = 0$$

all have negative real part, it is sufficient to assume merely that $\phi(t)$ and $\psi(t)$ are sufficiently small, depending upon A. It is more than enough that they tend to zero as $t \to \infty$. If we impose this restriction on A, and the condition on the f_i that they are power series in the y_k, with constant coefficients, beginning with quadratic terms, we have a fundamental theorem of Liapounoff. It does not seem to have been previously noticed that the hypothesis of Cesari's theorem (essentially (2) of Theorem 1) can be weakened to a boundedness condition if the above restriction is made upon A.

We shall sketch the method of proof. The differential equation (4) is transformed into the integral equation

$$y = x + \int_0^t X(t)X^{-1}(t_1)f(y(t_1),t_1)dt_1, \tag{8}$$

where $X(t)$ is the matrix solution of (3) satisfying $X(0) = I$.

If A is constant, the equation is even simpler, and becomes

$$y = x + \int_0^t X(t - t_1)f(y(t_1),t_1)dt_1. \tag{9}$$

The conditions imposed on $f(y, t)$ and A are now sufficient to show convergence of the sequence

$$y_0 = x$$
$$y_{n+1} = x + \int_0^t X(t)X^{-1}(t_1)f(y_n(t_1), t_1)dt_1 \qquad (10)$$

using the classical methods.

Theorem 3 requires use of the Birkhoff-Kellogg[2] fixed point theorem applied to (8).

The Liapounoff result can be proved using the character of the solution of (3), for A restricted as above, and the quadratic character of the f_i.

2. Using the elementary methods mentioned previously, the following theorem can be obtained:

THEOREM 4: *All solutions of*

$$\frac{d^2x}{dt^2} = (A + B)x$$

are bounded, provided

(1) A is symmetric with negative characteristic roots.
(2) $\|B\| = (\sum_{ij} |b_{ij}|^2)^{1/2} \leq b.$
(3) $\int^\infty \left\| \frac{dB}{dt} \right\| dt < \infty.$

The constant b depends upon A.

COROLLARY: *All solutions of*

$$\ddot{y} + (a^2 + \phi(x))y = 0$$

are bounded, if

(1) $\int^\infty |\phi'(x)|dx < \infty.$
(2) $|\phi(x)| \leq b < a^2.$

[1] Bellman, R., *Duke Math. Jour.*, **10**, 643–648 (1943).
[2] Birkhoff, G. D., Kellogg, O. D., *Trans. Amer. Math. Soc.*, **23**, 96–115 (1922).
[3] Cesari, L., *Ann. Scuola norm. super. Pisa*, **8**, 131–148 (1939).
[4] Liapounoff, S., *Annales de Toulouse*, **9**, 203–475 (1907).